


X34 staining

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 An abbreviated version of this protocol was published in eLIFE in May 2019

Intrinsically aggregation-prone proteins form amyloid-like aggregates and contribute to tissue aging in *Caenorhabditis elegans*

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Detailed protocol

X34 staining to detect amyloid structures in *C. elegans*:

1. Dissolve X-34 from Sigma (SML1954) into DMSO at 25 mg/ml. From this stock solution, make 1 mM X-34 in 10 mM Tris-HCl (pH 8).
1. Place 50 adult *C. elegans* of the same age into an Eppendorf tube with 1 ml M9 with 0.01% Triton.
2. Vortex briefly at low speed and spin down in mini-centrifuge at low speed. Remove supernatant. If bacteria still present, wash again with 1ml M9 with 0.01% Triton.
3. Remove supernatant and add 100 µl of 1 mM X-34. Place on nutator (in the dark) and gently shake at room temperature for two hours.
4. Spin down in mini-centrifuge at low speed and remove supernatant. To worm pellet, add 100 µl M9 with 0.01% Triton. Pipette worms onto seeded NG plate (cut end of pipette tip to enlarge opening). Let dry and leave the worms to destain overnight.
5. Next day, prepare fresh 2% agarose pad and pick destained worms into 15 µl M9 with 2 µM levamisole for anaesthesia.
6. Proceed to imaging with confocal microscope using 405 nm for excitation and an emission range between 470-520 nm.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. David, D. C. (2020). X34 staining. Bio-protocol Preprint. bio-protocol.org/prep328.
2. Huang, C., Wagner-Valladolid, S., Stephens, A. D., Jung, R., Poudel, C., Sinnige, T., Lechler, M. C., Schlörit, N., Lu, M., Laine, R. F., Michel, C. H., Vendruscolo, M., Kaminski, C. F., Kaminski Schierle, G. S. and David, D. C. (2019). Intrinsically aggregation-prone proteins form amyloid-like aggregates and contribute to tissue aging in *Caenorhabditis elegans*. eLIFE. DOI: [10.7554/eLife.43059](https://doi.org/10.7554/eLife.43059)

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